Neuroscience Letters 450 (2009) 147–151

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Attenuation of epilepsy-induced brain damage in the temporal cortices of rats by exposure to LTP-patterned magnetic fields

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ARTICLE INFO

Article history:
Received 6 August 2008
Received in revised form 31 October 2008
Accepted 4 November 2008

Keywords:
Long-term potentiation
Temporal cortices
Hippocampus
Cell damage
Physiologically patterned magnetic fields
Rat
Seizures

ABSTRACT

To test the feasibility that whole body exposure to structurally matched, physiologically patterned magnetic fields could reduce cellular injury within specific regions of the brain, young rats were seized with lithium and pilocarpine and then exposed to a sham field or to one of three computer-generated magnetic field patterns. They were digitized equivalents of the pulsed patterns of electric current known to produce long-term potential (LTP) in slices of hippocampus or entorhinal cortices. Histological analyses of their brains as adults indicated the earlier exposure to the LTP-patterned fields produced a robust reduction of damage within the primary and association areas of the right temporal cortices and the CA1/CA2 hippocampal fields. The results suggest physiologically patterned magnetic fields could be employed to target specific nuclei anywhere within the brain by matching intrinsic activity.
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Neuronal dropout from the myriad sources of brain injury alters behaviour permanently. Although there are many pharmacological methods for reducing brain damage, their efficacy is limited by vascular distribution and the availability of receptor subtypes on cell surfaces. Whole brain applications of physiologically patterned magnetic fields during disruptive physiological conditions have been shown to reduce cell damage [15] and to increase the rate of recovery of crushed peripheral nerves [18]. Recently a series of elegant experiments [2] showed that a pulsed, physiologically patterned magnetic field produced robust and reliable increased amplitudes of population spike potentials in hippocampal slices. Sequential experiments indicated a significant role of electrical synapses which connect neurons into functional networks.

Exposure of adult rats to an "amygdaloid" burst-firing pattern during the initial stages of lithium/pilocarpine-induced seizures, known to shift more than half of hippocampal neurons from normal to burst-firing profiles [20] through permanent up-regulation of T-type calcium channels, can enhance later spatial learning [5]. We present here evidence that whole body application of specific patterns of weak magnetic fields known to induce long-term potentiation (LTP) when applied as electric current to the limbic region [16,22] can produce striking reductions in the amount of cell loss in specific regions of the temporal cortices and hippocampus.

Wide spread neuronal loss whose proportions vary from structure to structure occur primarily within the rat thalamus, limbic areas, and neocortices following the induction of limbic motor seizures by a single systemic injection of a therapeutic dosage of lithium (3 mequiv./kg) and pilocarpine (30 mg/kg) or a single much larger dosage (300 mg/kg) of pilocarpine [10,12]. The potential experimental "targeting" of specific regions within the cerebral volume to ameliorate neuronal damage by applied sinusoidal or square-wave magnetic fields has been limited by their diffuse effects [6,21].

One approach to overcome this limitation is to design complex, temporally patterned magnetic fields that are similar to specific neuroelectrical patterns typically and uniquely generated by cellular aggregates within specific regions of the brain [7] in order to produce "resonance" [2,7]. Yun et al. [22] found that different stimulation patterns were most effective for inducing LTP in layers I, II–III, and V of the entorhinal cortices (ECs). Input from the entire cortical mantle converges on superficial layers II and III of the EC. In turn EC layers II and III are the origin of the perforant pathway [3]. Layer II axons, comprising the most prominent component of this pathway, terminate on the molecular layer of the dentate gyrus and on the stratum lacunosum-moleculare of CA3.

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doi:10.1016/j.neulet.2008.11.019

We have found that 30 min whole body application of a simpler magnetic field pattern [8,19] after contextual fear training produced a conspicuous interference in subsequent memory. Similarly timed bursts of electric current applied to hippocampal slices evoke LTP. Sinusoidal waves of comparable frequency and intensity did not affect this behaviour.

To discern if physiologically patterned magnetic fields could attenuate seizure-induced brain damage, 21–22 old male Wistar albino rats (whose mothers had been obtained from Charles Rivers, Quebec) were injected subcutaneously with 3 mequiv./kg of lithium chloride and 4 h (between 1 and 2 h LT in red light) later with 30 mg/kg of pilocarpine [10]. Immediately after rearing and forelimb clonus (4–7 min for rats in each group), they were injected with 30 mg/kg of acepromazine (to facilitate survival) and placed in a plastic chamber equipped with six solenoids (small "reed switches") embedded in the center of each wall as described elsewhere [8]. The ±5 V transformations of numbers between 1 and 257 (<127 = negative polarity; >127 = positive polarity) was controlled by computer software; there were no sound artifacts. The 1 μT magnetic fields (as measured by a Metex 3800 multimeter and magnetic sensor probe from Electric Field Measurements) were generated every 0.5 s successively in each spatial plane (between opposite solenoids) and then in all three planes (2 s complete cycle). The intensity and frequency is sufficient to affect brain tissue [1]; slightly higher intensities have been shown to evoke electrical seizures in epileptic humans [5].

Rats were exposed either to the sham field or to one of three magnetic field patterns. The sham field involved exposure to the chamber with all equipment running except the interface between the digital-to-analogy converter was disconnected. The patterns were: (1) Rose's LTP [16], (2) Theta Burst Stimulation [22] (TBS) or (3) Sharp Waves Burst Stimulation [22] (SPWBS); they are shown in Fig. 1. These patterns reflect the actual shape of the applied field through the solenoids as discerned by direct measurement by placing a test solenoid within the exposure area. Rose's simple LTP was a series of 1 ms pulses followed 170 ms later by four successive

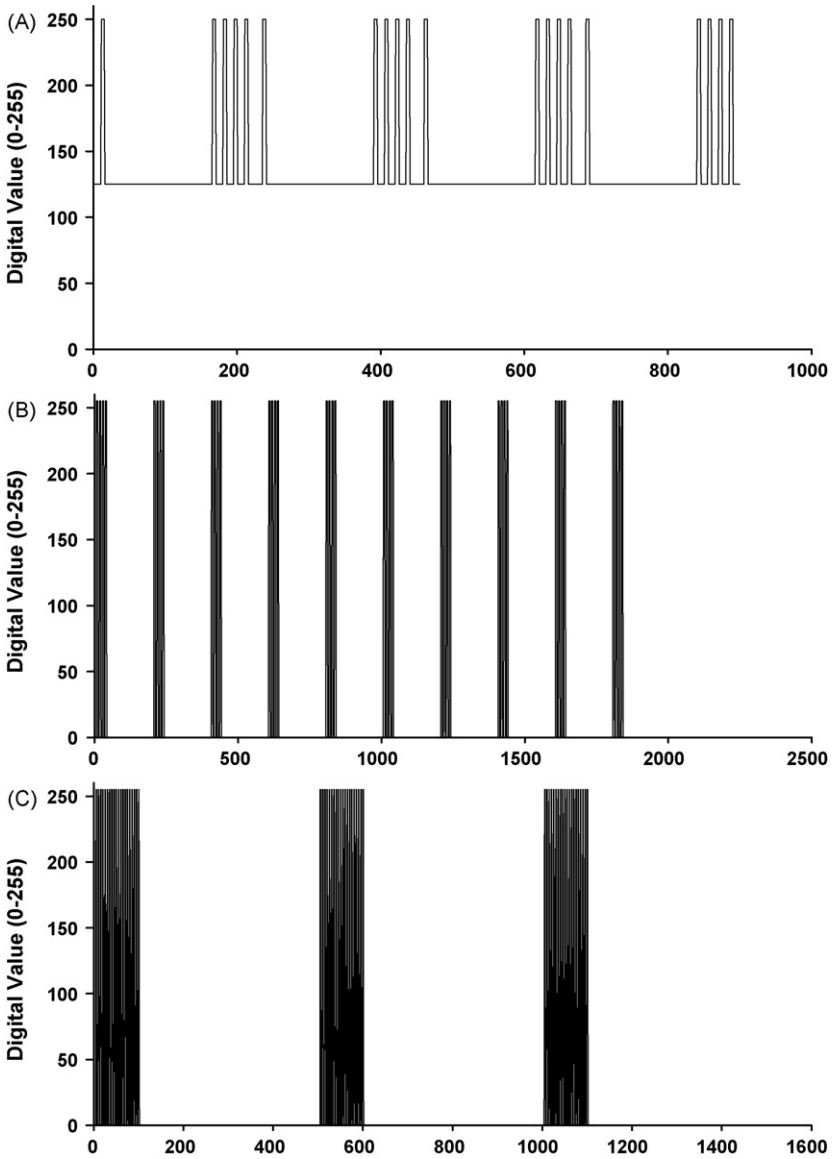

Fig. 1. Temporal patterns of 1 μT magnetic fields to which the rats were exposed for 1.5 h after induction of seizures by lithium/pilocarpine. (A) Rose et al.'s [16] LTP pattern, (B) Theta Burst Stimulation (TSB), (C) Sharp Wave Burst Stimulation (SPWBS). The latter two were modelled from Yun et al. [22]. The *x*-axis refers to time units (ms); *Y* refers to the values between 1 and 257 that reflected the −5 V to +5 V delivered to the solenoids. The Rose pattern is positive polarity only with no field between pulses. The individual pulses within the blocks in (C) may be obscured because of size reduction.

pulses. The field was only positive polarity; the zero value for the interstimulus interval indicated no magnetic field was being generated. The TBS pattern consisted of 10 high frequency bursts (four pulses at 100 Hz) that primarily affected layer II–III of the entorhinal cortices. The SPWBS consisted of three high frequency bursts (20 pulses at 200 Hz) repeated at 2 Hz that primarily affected layer V of the EC. All patterns were generated by digitizing the patterns into successive values between 0 and 255, with point durations, the time each voltage value was generated to a pair of solenoids, equal to 1 ms. The patterns were generated from custom software through a digital-to-analogue computer and then distributed to the pairs of solenoids within the chamber [8].

The total duration of exposure immediately following the injection of the acepromazine was 1.5 h. A total of 32 rats were exposed to one of these four conditions. The rats were then maintained three per cage for the rest of their lives. Food and water were available *ad libitum*. Ambient temperature was maintained at 20 °C and the 12:12 h light:dark cycle began at 08 h. At 120 days of age 12 of the rats were randomly selected from the pool of 32 rats and decapitated. Because our research focuses upon the long-term (adult) consequences of synergisms [21] between early extreme physiological conditions, such as seizures, and patterned magnetic fields, we waited until the rats were adults. The numbers of rats in each condition (in parentheses) were: sham ($n = 4$), Rose's LTP ($n = 3$), TBS ($n = 2$) and SPWB ($n = 3$). We reasoned that clinical utility requires large effect sizes and hence smaller samples.

The brains were removed, fixed in ethanol–formalin–acetic acid, embedded in paraffin and sectioned by microtome at 10 μm. Ten equally spaced sections (comparable for all rats) between the posterior and anterior commissures were stained with toluidine Blue O. They were examined by light microscopy at 100× and 400× for the proportion of damage (neuronal dropout) according to established methods [10,12,13] for the left and right side the brain for the major 78 differentiations for the cerebral cortices, amygdala, and hippocampus as indicated by Paxinos and Watson [9]. Essentially the proportion of damage (0–10) of the presence or absence (0,1) of conspicuous damage within each structure was recorded for each section and averaged for that structure. The values from this method have been demonstrated to be correlated strongly ($\rho = 0.78$) with numerical discrepancy between cell density in specific nuclei for seized compared to normal rats [10] and (>0.90) with actual behavioural deficits associated with the specific regions [12].

We predicted the most significant attenuation should occur in the right hemisphere compared to the left. The number of spontaneous seizures [11] during the year following lithium/pilocarpine-induced epilepsy is most correlated with neuronal loss in the right temporal cortices [13]. Second, we predicted that the most significant attenuation of damage from this 1.5 h, post-seizure exposure to this specific physiological pattern of magnetic fields should occur within the hippocampus, entorhinal cortices, and temporal lobes because of their mutual interconnections. All analyses involved SPSS software on a VAX 4000 computer.

The most robust treatment effects were observed within the primary (Te1) and association (Te3) auditory components of the temporal cortices. Because there were no significant differences between the TBS and SPWB groups, their data were combined. The means and standard errors of the means are shown in Fig. 2. Analyses of variance indicated that the rats that had been exposed to the complex LTP patterns showed a near-elimination of neuronal damage within the Te1 region of the right temporal cortices [$F(2,9) = 30.49$, $p < 0.001$; ω^2 estimate >0.90] compared to sham-exposed rats while the simple LTP groups occupied an intermediate position. A similar effect was seen for the Te3 region of the right hemisphere. The effect was conspicuous by even visual inspection as seen in Fig. 3. A representative rat that was not treated displayed

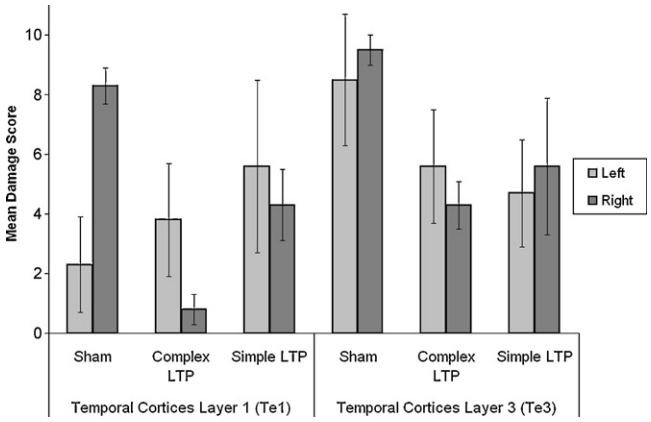

Fig. 2. Mean damage scores (10 = neuronal loss observed in all slides) within the left and right temporal cortices (Te1 and Te3) for adult rats that had received whole body exposure for 1.5 h as weanlings during seizure induction to either sham conditions or to the simple (Rose et al. [16]) or complex (Yun et al. [22]) LTP-patterned magnetic fields.

the usual paucity of neurons while two rats treated with either the simple or a complex LTP pattern showed marked neuronal savings. This relative reduction was not seen in the left hemisphere. Even the ratio of damage (right side/divided by left side) demonstrated this ameliorating effect [$F(2,9) = 5.08$, $p = 0.03$; $\omega^2 = 55\%$ of variance explained]. There were no significant treatment differences for the visual association cortices (Te2) or for any of the occipital, parietal, or frontal cortices.

The means and S.E.M.s for the proportion of damage within the left and right sides of the hippocampal formation (CA1, CA2, CA3, DG) and the entorhinal cortices are shown in Table 1. The rats that had received the 1.5 h of magnetic field stimulation by either the simple or complex LTP patterns during and after the seizure onset at 22 days of age showed statistically significant ($p < 0.01$) reduction of damage scores within the left and right CA and CA2 fields of the

Table 1
Means and standard errors of the mean (S.E.M.) for the proportion (out of 10) of damage within the left and right sides of the hippocampal and entorhinal cortical regions in adult rats following induction of limbic seizures at 22 days of age while being exposed to either sham fields or one of two types of magnetic fields (1 μT) designed to imitate LTP-inducing patterns for 1.5 h.

Structure	Sham field		Simple LTP		Complex LTP	
	M	S.E.M.	*M*	S.E.M.	*M*	S.E.M.
Entorhinal Ctx						
Left						
Lateral	10.0	0	10.0	0	9.6	0.4
Intermediate	10.0	0	10.0	0	9.6	0.4
Medial	10.0	0	9.3	0.3	8.2	0.6
Right						
Lateral	10.0	0	10.0	0	9.8	0.2
Intermediate	9.7	0.3	9.6	0.4	9.0	0.4
Medial	9.8	0.3	8.3	0.9	9.0	0.4
Hippocampus						
Left						
CA1	9.5a	0.5	5.7b	0.9	5.8b	0.9
CA2	6.8a	1.2	1.7b	0.9	2.2b	0.9
CA3	9.0	0.7	6.3	0.8	7.6	1.5
DG	8.8	0.2	7.0	1.5	7.4	0.9
Right						
CA1	9.0a	0.7	4.7b	1.3	6.2	0.9
CA2	8.5a	0.6	1.0b	1.0	1.8b	1.3
CA3	8.0	1.2	7.3	0.9	7.2	1.2
DG	8.5	0.9	5.6	1.0	6.0	1.0

a vs. b, $p < 0.05$.

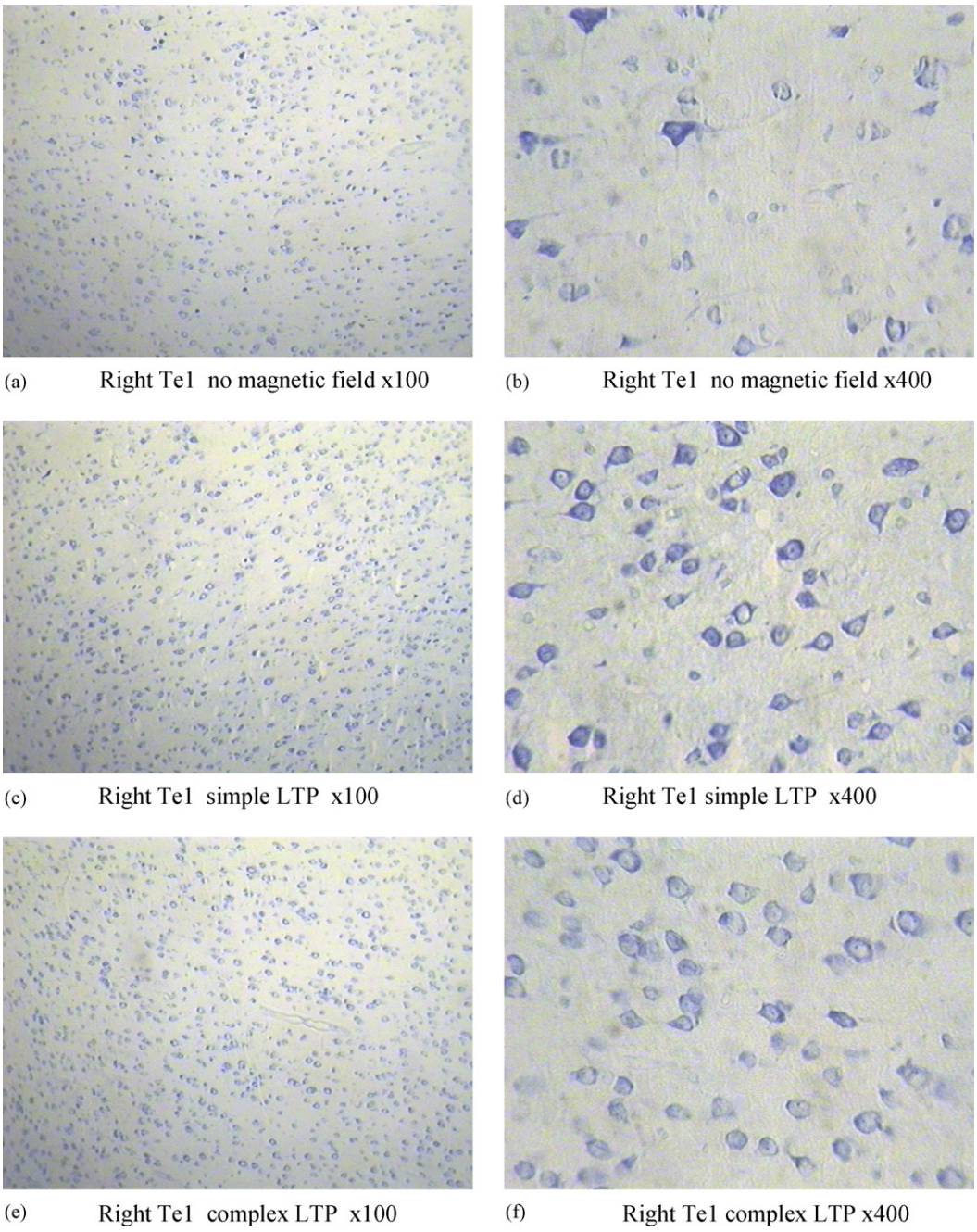
(a) Right Te1 no magnetic field x100
(b) Right Te1 no magnetic field x400
(c) Right Te1 simple LTP x100
(d) Right Te1 simple LTP x400
(e) Right Te1 complex LTP x100
(f) Right Te1 complex LTP x400

Fig. 3. Sample sections from the right Te1 sections of adult rats at 100× and 400× for rats that were seized but did not receive any treatment (A and B), received the simple [16] LTP magnetic field (C and D), or received one (Fig. 1C) of the complex LTP magnetic fields (E and F) during the induction of seizures as weanlings.

hippocampus. The amount of variance in these scores accommodated by the treatment was about 70%.

The results of this experiment clearly indicate that a brief whole body exposure to physiologically patterned magnetic fields for only 1.5 h during the initial stages of experimentally induced epilepsy in young rats can result in a robust reduction of damage within the auditory (Te1, Te3) components of the right temporal cortices and within the CA1 and CA2 fields of the hippocampus bilaterally. The complex LTP patterns, developed by Yun et al. [22] to induce LTP in various layers of the entorhinal cortices, were most effective for the temporal cortices while both the complex and simple LTP pattern, the latter developed by Rose et al. [16] for stimulation of hippocampal slices, were effective for reducing hippocampal damage.

The laterality (right side) effect for the temporal lobe is interesting in light of the strong correlation between measurements of damage (using a different method) within the Te1 and the number of spontaneous seizures observed during the year following the induction [13]. We suggest that the brief exposure to the LTP-patterned magnetic fields during this period of marked physiological heterostasis may have modified processes that prevented the development of these seizures and the correlative neuronal damage that would typically have been seen when the rats became adults.

The ameliorating effect of the simple LTP pattern, as well as the complex form, would be expected given its origin: to evoke LTP in hippocampus slices. The similarity of the strength of the effect for both simple and complex forms suggests that any mild discrepancy

in field strength was not the critical factor. It may be relevant that because the entorhinal cortices projects to both the hippocampus and Te1 and Te3 [17] the beneficial effects may occur only "down stream" from the focus of the massive damage. This experiment suggests that physiologically patterned magnetic field patterns can be targeted to affect specific brain regions with relatively unique electrophysiological signatures despite whole brain or whole body exposure.

Although there are many potential mechanisms the calculations and measurements of Pilla et al. [14] and Blank and Soo [4] are most relevant. The former found that pulsed magnetic field effects on Ca^{2+}/calmodulin-dependent myosin phosphorylation occurred during nonequilibrium phases of reactions (which characterize physiological extremes). The rate constant of the binding process involved with magnetic field sensitivity is about 1.5 ms while the dissociation of Ca^{2+} from CaM is bimodal is between 25 and 100 ms or 2 and 3.3 ms. They [14] concluded that Ca^{2+} dissociation from low-affinity binding site is the electromagnetic field-sensitive step. Blank and Soo [4] found that 50 Hz magnetic fields below 0.3 μT increased activity of Na,K-ATPase and the rate constant of cytochrome oxidase when the electron flow was below 10^{16} electrons/(mg protein min). Disruption of Ca^{2+} binding (and downstream apoptosis/necrosis) and enhanced CYO and Na,K-ATPase from the temporally patterned magnetic fields during the extreme physiological intracellular states associated with the seizure may have facilitated cell survival.

References

[1] W.R. Adey, Frequency and power windowing in tissue interaction with weak electromagnetic fields, Proc. IEEE 68 (1980) 119–125.
[2] Z. Ahmed, A. Wieraszko, The mechanism of magnetic field-induced increase of excitability in hippocampal neurons, Brain Res. 1221 (2008) 30–40.
[3] A. Alonso, R. Klinik, Differential responsiveness of stellate and pyramidal-like cells in the medial entorhinal cortex Layer II, J. Neurophysiol. 70 (1993) 128–143.
[4] M. Blank, L. Soo, Enhancement of cytochrome oxidase activity in 60 Hz magnetic fields, Bioelectrochem. Bioenerg. 45 (1998) 253–259.
[5] L.L. Cook, M.A. Persinger, Long-term consequences of subtle stimuli during the first twenty-four hours of seizure-induced brain injury, Percep. Mot. Skills 83 (1996) 523–529.
[6] J. Dobson, T. St-Pierre, H.G. Wieser, M. Fuller, Changes in paroxysmal brain wave patterns of epileptics by weak-magnetic field stimulation, Bioelectromagnetics 21 (2000) 94–99.
[7] L.J. Martin, S.A. Koren, M.A. Persinger, Thermal analgesic effects from weak, complex magnetic fields and pharmacological interactions, Pharm. Biochem. Behav. 78 (2004) 217–277.
[8] B.E. McKay, M.A. Persinger, S.A. Koren, Exposure to a theta-burst patterned magnetic field impairs memory acquisition and consolidation for contextual but not discrete conditioned fear in rats, Neurosci. Lett. 292 (2000) 99–102.
[9] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates, 2nd ed., Academic Press, Orlando, FL, 1986.
[10] O. Peredery, M.A. Persinger, G. Parker, L. Mastrosov, Temporal changes in neuronal dropout following inductions of lithium/pilocarpine seizures in the rat, Brain Res. 881 (2000) 9–17.
[11] M.A. Persinger, G. Belanger-Chellew, Brief successive temporal observational sampling as possible indicator of daily overt seizure activity in epileptic rats, Percep. Mot. Skills 92 (2001) 95–98.
[12] M.A. Persinger, Y.R.J. Bureau, O. Peredery, Dissociation between conditioned taste aversion and radial maze learning following seizure-induced multifocal brain damage: quantitative tests of serial vs. parallel circuit models of memory, Physiol. Behav. 56 (1994) 225–235.
[13] M.A. Persinger, M.J. Dupont, Emergence of spontaneous seizures during the year following lithium/pilocarpine-induced epilepsy and neuronal loss within the right temporal cortices, Epilep. Behav. 5 (2004) 440–445.
[14] A.A. Pilla, D.J. Muehsam, M.S. Markov, B.F. Sisken, EMF signals and ion/ligand binding kinetics: prediction of bioeffective waveform parameters, Bioelectrochem. Bioenerg. 48 (1999) 27–34.
[15] J.A. Robertson, A.W. Thomas, Y. Bureau, F.S. Prato, The influence of extremely low frequency magnetic fields on cytoprotection and repair, Bioelectromagnetics 28 (2008) 16–30.
[16] G.M. Rose, D.M. Diamond, K. Pang, T.V. Dunwiddie, Primed burst potentiation: lasting synaptic plasticity invoked by physiologically patterned stimuli, in: H.L. Haas, G. Buzaki (Eds.), Synaptic Plasticity in the Hippocampus, Springer-Verlag, Berlin, 1988, pp. 96–98.
[17] J.A. Rosenkranz, A.A. Grace, Modulation of basolateral amygdala neuronal firing and afferent drive by dopamine receptor activation in vivo, J. Neurosci. 19 (1999) 11027–11039.
[18] B.F. Sisken, J. Walker, M. Orgel, Prospects of clinical applications of electrical stimulation for nerve regeneration, J. Cell. Biochem. 51 (1993) 404–409.
[19] L.S. Stewart, M.A. Persinger, Pretraining exposure to physiologically patterned electromagnetic stimulation attenuates fear-conditioned analgesia, Int. J. Neurosci. 100 (2000) 91–98.
[20] H. Su, D. Sochivko, A. Becker, J. Chen, Y. Jiang, Y. Yaari, H. Beck, Upregulation of T-type Ca^{2+} channel causes a long-lasting modification of neuronal firing mode after status epilepticus, J. Neurosci. 9 (2002) 3645–3655.
[21] P.M. Whissell, M.A. Persinger, Emerging synergism between drugs and physiologically-patterned weak magnetic fields: implications for neuropharmacology and the human population in the twenty-first century, Curr. Neuropharm. 5 (2007) 278–288.
[22] S.H. Yun, I. Mook-Jung, M.W. Jung, Variation in effective stimulus patterns for induction of long-term potentiation across different layers of rat entorhinal cortex, J. Neurosci. 22 (2002), RC214 1 of 5.